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Activation of neuromedin B-preferring bombesin receptors on rat glioblastoma C-6 cells increases cellular Ca2+ and phosphoinositides.

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Recent cloning studies confirm the presence of two subtypes of bombesin (Bn) receptors. In contrast to the gastrin-releasing peptide (GRP)-preferring subtype, which has been widely studied, nothing is known about the cellular mechanisms of the neuromedin B (NMB)-preferring subtype, which occurs widely in the central nervous system and gastrointestinal tissues, partially because of the lack of a cell line with functional receptors. In the present study we have investigated Bn receptors on the rat glioblastoma cell line C-6, reported to contain mRNA of the NMB receptor subtype. Binding of 125I-[D-Tyr0]NMB to these cells was time- and temperature-dependent, saturable, reversible, and only inhibited by Bn receptor agonists or antagonists. For Bn receptor agonists the relative potencies were: NMB (1.7 nM) approximately equal to litorin (3 nM) greater than ranatensin (8 nM) greater than Bn (19 nM) greater than neuromedin C (NMC) (210 nM) greater than GRP (500 nM). These relative affinities were almost identical to those for the NMB receptor subtype on rat oesophageal tissue and for Balb 3T3 cells stably transfected with the NMB receptor subtype. These potencies differed from those for the GRP receptor subtype on rat pancreatic acini [Bn approximately equal to litorin (4 nM) greater than ranatensin, NMC, GRP (15-20 nM) much greater than NMB (351 nM)]. The relative potencies of four different classes of Bn receptor antagonists were compared. Results from C-6 tumour cells agreed closely with those for binding to the NMB receptor subtype on rat oesophageal tissue and in Balb 3T3 cells stably transfected with this receptor, and differed markedly from those for binding to the GRP receptor subtype on rat pancreatic acini. Four Bn receptor antagonists had a higher affinity for the GRP subtype ([D-Phe6]Bn-(6-13)ethyl ester (500 x), [D-Phe6][psi 13-14,Cpa14]Bn- (6-14) (70 x) (where psi 13-14 refers to the replacement of the -CONH- peptide bond between Leu13 and Met14 by -CH2NH2) [psi 13-14,Leu14]Bn, [D-Phe6]Bn-(6-13) propylamide (30 x)] and two had a higher affinity for the NMB subtype on C-6 cells and transfected cells ([D-Pro4,D-Trp7,9,10] substance P-(4-11) (9 x) and [Tyr4,D-Phe12]Bn (18 x)]. In C-6 tumour cells, Bn receptor agonists caused an increase in cytosolic Ca2+ and the generation of inositol phosphates. For both responses, NMB was more than 50-fold more potent than GRP. Neither NMB nor GRP increased cyclic AMP. These results demonstrate that the rat glioblastoma cell line C-6 possesses functional NMB-preferring Bn receptors, and agonist occupation activates phospholipase C, thus increasing cytosolic Ca2+ and inositol phosphate formation. Because the interaction of Bn-related peptides with C-6 cell receptors is identical with that reported in other tissues containing the mRNA for the NMB subtype, this cell line should prove useful in exploring further the cellular basis of action of the peptides that interact with this receptor in the central nervous system and various other tissues.